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Phytofiltration of hazardous cadmium, chromium, lead and zinc ions by biomass of *Medicago sativa* (Alfalfa)

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Abstract

Previous laboratory batch experiments of *Medicago sativa* (Alfalfa) indicated that the African shoots population had an appreciable ability to bind copper(II) and nickel(II) ions from aqueous solution. Batch laboratory pH profile, time dependency and capacity experiments were performed to determine the binding ability of the African shoots for cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II). Batch pH profile experiments for the mentioned ions indicated that the optimum pH for metal binding is approximately 5.0. Time dependency experiments for all the metals studied showed that metal binding to the African alfalfa shoots occurred within 5 min. Binding capacity experiments revealed the following amounts of metal ions bound per gram of biomass: 7.1 mg Cd(II), 7.7 mg Cr(III), 43 mg Pb(II), and 4.9 mg Zn(II). However, no binding occurred for chromium(VI). Nearly all of the metals studied were recoverable by treatment with 0.1 M HCl. Column experiments were performed to study the binding of Cd(II), Cr(III), Cr(VI), Pb(II) and Zn(II) to silica-immobilized African alfalfa shoots under flow conditions. These experiments showed that the silica immobilized African alfalfa shoots were effective for removing metal ions from solution, and over 90% of the bound Pb(II), Cu(II), Ni(II), and Zn(II), and over 70%Cd(II), were recovered after treatment with 10 bed volumes of 0.1 M HCl. The results from these studies will be useful for a novel phytofiltration technology to remove and recover heavy metal ions from aqueous solution. © 1998 Elsevier Science B.V.

Keywords: Phytofiltration; Alfalfa; *Medicago sativa*; Heavy metal binding; Recovery

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1. Introduction

The accumulation of heavy metal contaminants in the environment has become a concern due to the growing health risks to the public. These contaminants, such as cadmium, chromium, lead and zinc, enter the environment through industrial waste, mill tailings, and landfill runoff. Exposure to heavy metal ion contamination has been found to cause kidney and liver damage, and anemia in low doses. In high concentrations, heavy metals can be carcinogenic and teratogenic, if not fatal [1]. The US Environmental Protection Agency has regulated contaminant industrial waste disposal; however, when industrial waste discharges exceed the regulated disposal concentrations, many industries respond by diluting the hazardous materials to be within the allowable limits. Once the diluted hazardous substances are released into the environment, they naturally concentrate into wetlands and soils. The natural processes of transportation of metal ions between the soil and water consolidate heavy metal contamination in high concentrations that affect the natural ecosystems [2]. Because of the increasing environmental concern regarding heavy metal contamination, there has been a great deal of interest in the removal of heavy metal ions from contaminated soils and waste streams [3–8]. Although cleanup is necessary, to prevent any further discharge of contaminated wastes into the environment, a technology needs to be developed that is cost-effective for industry to use. Methods traditionally employed for water remediation consist of removal of heavy metals by filtration, flocculation, activated charcoal, and ion-exchange resins. However, because of the high cost of these methods, the development of a more cost-effective remediation system is necessary.

There has been a tremendous amount of attention given to the use of biological systems for the removal of heavy metal ions from contaminated areas [9–14]. More recently, phytoremediation has emerged as one of the alternative technologies for removing pollutants from the environment. Interest in using plants for environmental remediation is increasing due to their natural capacity to accumulate heavy metals, and degrade organic compounds [15–17]. Since chemical functional groups are most likely responsible for the metal binding, it is likely that higher plant cells might also be capable of metal binding. Various research groups have conducted studies on the metal binding properties of plant tissues [18–22]. Investigations performed by the genetic research group at Los Alamos National Laboratories have shown binding of cadmium(II) by cells of *Datura innoxia* [23]. Plant species that have been found growing on heavy-metal-contaminated soils have become tolerant to the toxic effects of heavy metals [24,25]. This heavy metal tolerance by the plants may be due to the evolution of chemical functional groups that reduce the toxic effects of the heavy metals. Therefore, plants may be a good source for naturally occurring biological compounds that may have the potential for heavy metal contamination removal from waste water.

Alfalfa (*Medicago sativa*) has been found growing in soils being irrigated by heavy metal contaminated waters [24,25]. It has been reported that alfalfa has the ability to accumulate concentrations of heavy metals well above the tolerance levels of other plants [26–30]. This ability may be due to specialized chemical functional groups that could be responsible for metal tolerance and accumulation [31]. Because alfalfa possesses these chemical characteristics and is inexpensive and easily obtained, it may be a

potential biosource for the extraction and recovery of heavy metals from contaminated waters. The metal ions that were used for this study were chosen because of their threat to public health and the environment. Each of the metal ions studied are found in high concentrations at US EPA Superfund sites in Texas. Since it is the goal of the project to remove metal contaminants from water, it was essential to choose metals that are actually found at contaminated sites. For these reasons, we chose to study cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II), due to their toxic characteristics and actual concentrations in the environment.

The objective of this study was to investigate the binding of cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II), to African alfalfa shoots. Batch laboratory pH profile, time dependency and capacity experiments were performed to determine the binding ability of the African shoots to the above-mentioned metals. In addition, column experiments were performed with silica-immobilized African alfalfa shoots to determine the extraction and recovery ability of cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II) under flow conditions.

2. Methodology

2.1. Alfalfa collection

Alfalfa plants were collected from field studies conducted by Dr. John Henning at New Mexico State University near Las Cruces, New Mexico. The plants were removed from the soil, washed, and the roots were separated from the shoot material (stems and leaves). All samples were oven-dried at 90°C for one week. Dried samples were then ground to pass through a 100-mesh screen using a Wiley mill.

2.2. pH profile studies for metal binding

This experiment was carried out using the pH profile method previously reported by Gardea-Torresdey et al. [32]. In summary, a 250-mg sample of biomass was washed twice with 0.01 M hydrochloric acid (HCl) to remove any debris or soluble biomolecules that might interact with metal ions. Each biomass sample was resuspended in 50 ml of 0.01 M HCl with tissue concentration of approximately 5 mg per ml solution. A metal solution of 0.1 mM were prepared for the following metal ions: cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II) and the pH adjusted to 2.0, 3.0, 4.0, 5.0, and 6.0. Solutions were prepared from the corresponding salts: $Cd(NO_3)_2$, $Cr(NO_3)_3$, $K_2Cr_2O_7$, $Pb(NO_3)_2$, and $ZnCl_2$. At each pH (2.0, 3.0, 4.0, 5.0, and 6.0), 2 ml of the metal solution were added to the respective pH biomass pellet, and to the respective separated supernatant solutions. This was carried out 3 times for quality control. In addition, at each respective pH, 2 ml of the 0.1 mM metal solution were transferred to 3 tubes for controls. All the tubes were equilibrated on a rocker for 1 h. The samples were then centrifuged at 3000 rpm for 5 min and the supernatants from the pellets were transferred to clean tubes. Final pH values for all tubes were recorded, and analyses for metal ions were performed by flame atomic absorption spectroscopy.

2.3. Time dependence studies for metal binding

The time dependence batch experiments were performed using a procedure reported previously [32]. The procedure was followed to yield a concentration of biomass of approximately 10 mg per ml of solution. The solution was then adjusted to pH 5.0 and allowed to equilibrate. The time intervals chosen for the time dependence studies were: 5, 10, 15, 20, 25, 30, 45, and 60 min. This was carried out 3 times for quality control. After centrifugation and decantation, 2 ml of 0.3 mM metal solution were added to each of the tubes and controls. This procedure was repeated for each different metal ion being analyzed. Final pH values for all tubes were recorded and metal concentrations were determined by flame atomic absorption spectroscopy.

2.4. Metal binding capacity studies

The batch laboratory methods used to determine the binding capacity of Cd(II), Cr(III), Cr(VI), Pb(II), and Zn(II) to the African shoots were performed as reported previously for the binding of copper and nickel to *Medicago sativa* [32,33]. For these experiments, 100 mg of biomass were washed twice with 0.01 M HCl and washings were collected and weighed to determine any biomass loss. Washed biomass was resuspended in 10 ml of deionized water, and the pH was adjusted to 5.0. This was repeated for each one of the metal ions being studied, using 0.3 mM concentration of each metal ion at pH 5.0 in 0.01 M sodium acetate buffer. Final pH values for all tubes were recorded. Samples were diluted as required to remain within the calibration linear range, and metal concentrations were determined by flame atomic absorption spectroscopy.

2.5. Desorption of the adsorbed metal ions

To remove the bound metal ions from the alfalfa biomass, the pellets from binding capacity studies, with the adsorbed metal, were exposed to 2 ml of 0.1 M HCl. The samples were then centrifuged and the supernatants were removed as indicated by Gardea-Torresdey et al., [32]. The resulting supernatant solutions were collected for analyses and diluted as required to stay within the calibration range. Pellets were then exposed to 2 ml of 1 M HCl to remove any remaining metal and equilibrated by rocking for 5 min. After centrifugation, the supernatant solutions were analyzed. All metal analyses were performed by flame atomic absorption spectroscopy.

2.6. Immobilized alfalfa biomass and column experiments

The immobilization of the African alfalfa biomass was performed as indicated previously by Gardea-Torresdey et al. [34,35]. Samples of 5 g were washed twice with water, and the cell debris were removed by centrifugation. The following part of this experiment is similar to that reported before for the binding of copper and nickel to different species of *Medicago sativa* [32,33]. Seventy-five ml of 5% sulfuric acid (H_2SO_4) was mixed with enough 6% sodium silicate (Na_2SiO_3) solution to raise the pH

to 2.0. Once the solution was at pH 2.0, 5 g of washed biomass were added to the silica solution and allowed to stir for 15 min. The pH was then raised slowly by addition of 6% Na_2SiO_3 to reach a final pH of 7.0. The polymer gel with the immobilized biomass was dried overnight at 60°C and then ground by mortar and pestle and sieved to pass 20–40 mesh size. The metals studied were cadmium(II), chromium(III), chromium(VI), lead(II), and zinc(II). The metal solutions were passed through the column and the effluents were analyzed for metal content. One bed volume of solution that is passed through the column is equivalent to the volume of immobilized biomass within the column. In this case, the volume of immobilized biomass used was 6 ml; therefore, one bed volume is equal to 6 ml. The metal solutions were passed at a flow rate of 2 ml per min.

2.7. Recovery of metal ions from columns

To remove the bound metal from the immobilized African alfalfa shoots, 10 bed volumes of 0.1 M HCl were passed through the column at a flow rate of 2 ml per min. Each effluent bed volume was collected and analyzed by flame atomic absorption spectroscopy. The amount of metal recovered in each bed volume of effluent was summed, and the total was taken to be the total amount of metal recovered from the column.

2.8. Metal analyses

The metal content in all the experiments was performed by using a Perkin–Elmer model 3110 Atomic Absorption Spectrometer with deuterium background subtraction. The instrument response was periodically checked with known standards. A calibration curve was obtained with a correlation coefficient of 0.98 or greater. The samples were read three times and the mean value, as well as the relative standard deviation, were computed. Samples were diluted as required to remain within the calibration linear range. The following wavelengths were used for the metal ions studied: cadmium 228.8 nm; chromium 358.2 nm; lead 283.3 nm; zinc 213.9 nm. An impact bead was utilized to improve the sensitivity, but in the case of zinc a flow spoiler was used. Confidence intervals of 95% were calculated for each set of samples to determine the margin of error. The difference between the initial metal ion concentration and the remaining metal ion concentration was assumed to be bound to the biomass.

3. Results and discussion

Fig. 1 shows the binding of cadmium(II), lead(II), zinc(II), chromium(III), and chromium (VI) to African alfalfa shoots as a function of pH. Binding of every metal ion by the alfalfa biomass was unique. It can be observed that as the pH was increased, the amount of metal ion bound also increased, with most of the binding of zinc(II), lead(II), chromium(III) and cadmium(II) occurring between pH 5 and 6. It can also be observed from Fig. 1 that lead binding even occurred at pH 2. Chromium(III) and chromium(VI)

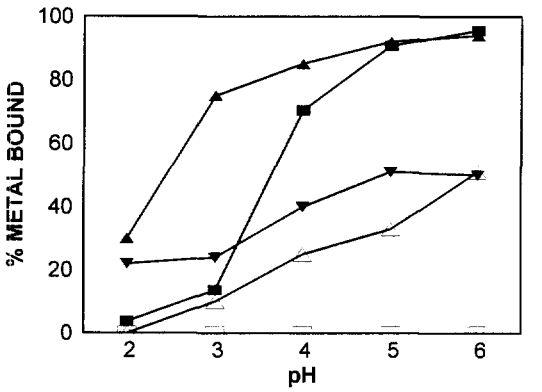

Fig. 1. Effect of pH on the binding of cadmium(II) ■; chromium(III) ▼; chromium(VI) □; lead(II) ▲; Zinc(II) △, by African Alfalfa shoots. Biomass (5 mg/ml) was shaken for 1 h at the appropriate pH with 0.1 mM of each of metal ion, independently.

were studied because of their different oxidation states that exist in contaminated waters, and differences in chemical properties. Chromium(VI) had no binding to the biomass because chromium(VI) exists in aqueous solution as oxo-anion (CrO_4^{-2} or $Cr_2O_7^{-2}$) with an overall charge of -2. If there is an electrostatic interaction between the metal ion and the alfalfa biomass, a negatively charged ion will not bind to a negatively charged ligand. This could be the case if the carboxylate groups are the binding sites on the alfalfa biomass, as it has been shown with other biomasses [36]. Therefore, the binding sites on the African shoot biomass responsible for this heavy metal ion uptake may only bind positively charged ions. This trend in pH suggests that these metal ions bind by a similar ion-exchange mechanism as observed with copper and nickel [32–35]. The pH-dependent trend also suggests that those metal ions that can be bound by African shoots may also be recovered by reducing the pH.

Fig. 2 demonstrates that the heavy metal ion uptake by the African shoots is very rapid for all the metal ions studied, with the exception of Cr(VI), for which no binding occurred. Metal ion binding occurred within 5 min and remained relatively stable thereafter. This trend was observed for all the metal ions under investigation, except Cr(VI). Due to the extensive prior washings, soluble compounds are eliminated as a possible means for heavy metal ion adsorption. The rapid uptake of Cd(II), Cr(III), Pb(II), and Zn(II) from solution suggests that the binding sites may be cell wall components, and that the metal ions are not diffusing through the cell wall. Since the tissues of the alfalfa plants studied were inactivated, we expect that rapid binding of Cd(II), Cr(III), Pb(II), and Zn(II) is due to the plant cell wall functional groups. Further studies will be performed to give us more information about the binding that is occurring, and to determine the binding constants as well as the binding mechanism of the metal ions.

Table 1 exhibits the amount of Cd(II), Cr(III), Cr(VI), Pb(II), and Zn(II) that was adsorbed from solution as the saturation point was reached. These studies were performed at pH 5.0. The binding capacities of the different populations are given in mg

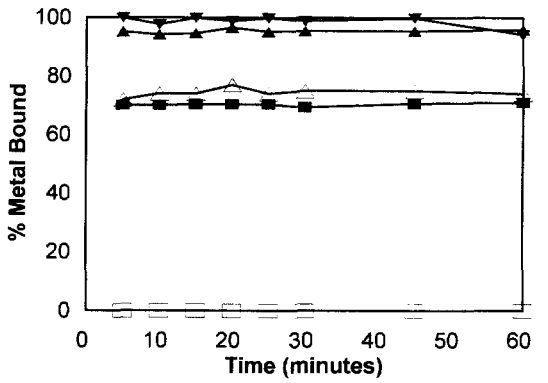

Fig. 2. Time dependency batch experiments for the binding of cadmium(II) ■; chromium (III) ▼; chromium (VI) □; lead (II) ▲; Zinc (II) △, by African Alfalfa shoots. Biomass was shaken for an appropriate time with 0.3 mM of each of metal ion, independently.

of metal adsorbed per gram of biomass. The range of adsorption for the metal ions studied was from 0 mg/g for Chromium(VI) to 43 mg/g for lead(II). The binding capacities obtained in these studies are comparable to those seen previously in other biomasses [20,32–35].

The pH profile experiments for heavy metal ion binding by the African shoots demonstrated that heavy metal ion uptake was minimum at low pH values. This suggested that we could remove the adsorbed metal ions by lowering the pH. Presumably, protons would then displace the adsorbed heavy metal ions. Table 2 shows the various percentages of metal ion recovered after reacting the metal ion bound alfalfa with 0.1 M HCl. As it can be seen in Table 2, recovery of Cd(II) and Pb(II) was more than 99%. In addition, almost 70% of the zinc was recovered from the biomass. However, the recovery for Cr(III) was only 13.9%. The reason for this low recovery is still not understood. By using acid at this low strength, the biomaterial is not destroyed and it can be reused. The reversibility of the heavy metal ion binding could have very important implications for the removal of metal ions from waste waters, since the metal ions can be adsorbed and also reclaimed. This may represent an innovative method for the removal and recovery of heavy metal ions from water by alfalfa biomass.

Table 1
Metal ion binding capacities of African Alfalfa shoots (batch experiments)

Metal ion	Capacity (mg/g)
Cadmium(II)	7.1
Chromium(III)	7.7
Chromium(VI)	0.0
Lead(II)	43.0
Zinc(II)	4.9

Table 2
Percentage recovery of metal ions bound to African Alfalfa shoots using 0.1 M HCl (batch experiments)

Metal ion	1% Recovery
Cadmium(II)	100.0
Chromium(III)	13.9
Chromium(VI)	0.0
Lead(II)	99.6
Zinc(II)	69.3

Our previous batch capacity experiments showed that African alfalfa shoots bind some heavy metal ions efficiently. However, it was necessary to conduct experiments with immobilized African shoots under flow conditions to determine if the immobilized alfalfa biomass could remove other metal ions from solution in a more practical way. It was necessary to use a silica polymer support matrix to immobilize the biomass to prevent the alfalfa cells from clumping together and reducing the effluent flow rate. Three cycles were performed with each column to maintain quality control. As seen in Fig. 3, the immobilized African shoots showed to be efficient in removing these metal ions from solution. The same molarity of metal ion solution was used for each of the metal ions studied (0.1 mM). Cadmium(II), lead(II), and zinc(II) showed to have binding capacities over 500 ppm. After 120 bed volumes of metal ion solutions had been passed through the column containing the immobilized alfalfa biomass, the highest capacity of all the metal ion binding was lead(II). The second closest was cadmium, followed by zinc(II) and chromium(III). After 120 bed volumes of metal ion solution had been passed, only chromium(III) was near saturation, the rest of the columns still had the ability to bind more metal ions. Chromium(VI) was not bound by the immobilized African shoots. We also used a control column, which contained only the polysilicate

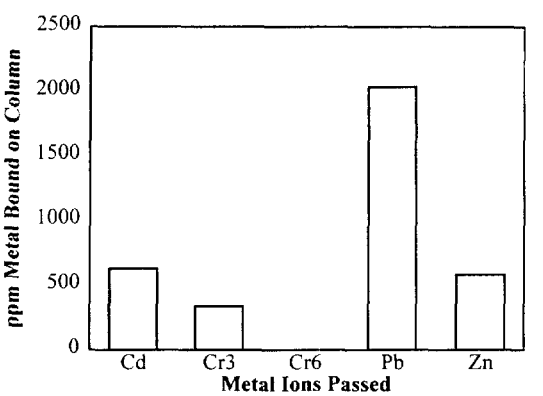

Fig. 3. Metal ions bound by immobilized African Alfalfa biomass in a column under flow conditions. After 120 bed volumes of 0.1 mM metal solution were passed, the effluents were analyzed for metal concentrations by flame atomic absorption. The x-axis represents the particular metal ion, and the y-axis represents the ppm of metal ion bound after 120 bed volumes were passed.

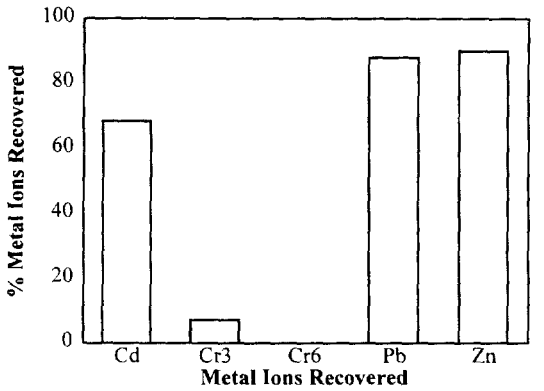

Fig. 4. Percentage of metal ion recovered from the column containing alfalfa biomass with metal bound. Ten bed volumes of 0.1 M HCl were passed through the column containing the particular bound metal. Effluents were analyzed for metal content by flame atomic absorption. The *x*-axis represents the particular metal ion, and the *y*-axis represents the percentage of metal ion desorbed.

support material that was used to entrap the biomass. The silica column did not bind any of the metal ions (data not shown). This indicates that the metal ion binding is due to a chemical interaction with the alfalfa biomass, and not by physical entrapment or chemical binding to the silica.

As indicated by the pH profiles, by using low-strength acid, the bound metal ions can be recovered from the immobilized African shoots. Fig. 4 shows the percentage of bound metal ions that were recovered after passing 10 bed volumes of 0.1 M HCl through the column. It can be seen that almost 90% of the lead(II), and zinc(II), were removed from the columns. For cadmium(II), recovery was almost 70%, and hardly any of the chromium(III) was recovered. Upon performing three cycles with the same column, some small changes were observed for the recovery percentages. The +2 charge ions showed to have good recoveries, which may be due to the metal ion displacement by protons. Chromium(III) may have been more tightly bound to its binding site, and it may be necessary to use a stronger acid concentration or other stripping agents to remove the metal ion. No chromium(VI) bound to the column, therefore, none was recovered. Since most of the metal ions that bound to the columns were recoverable, this could be an effective way to reclaim these metal ions from waste waters; however, other stripping agents need to be studied.

4. Conclusions

It was determined that the binding of Cd(II), Cr(III), Pb(II), and Zn(II) to African alfalfa shoots is pH-dependent, with most of the metal ion binding occurring around pH 5.0. Furthermore, experiments showed that most of the binding occurred within less than 5 min. In addition, metal ion binding remained constant throughout longer periods of time, without any desorption occurring. The fast binding of the heavy metal ions studied

suggests that most of the metal ion binding occurs on the cell walls of the African shoots. The lack of Cr(VI) binding leads us to believe that the adsorption occurs through an ion-exchange mechanism. Capacity and recovery batch experiments showed that African alfalfa shoots have an appreciable ability to uptake considerable concentrations of heavy metal ions. The recovery of the heavy metal ions is very feasible since over 99% of lead(II) and cadmium (II) was removed from the alfalfa biomass in the batch experiments. It was also shown that the adsorption and recovery of heavy metal ions from immobilized alfalfa shoots under flow conditions was as successful as in the batch experiments. The fact that the alfalfa shoots have the same efficiency under flow conditions suggests that they can be used in a more practical and economical method. Furthermore, the column experiments showed that the immobilized biomass is reusable. After they have been stripped of the heavy metal ion adsorbed, the columns containing the immobilized alfalfa biomass can be recycled.

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